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Tale of two transcription factors: NF- κ B and HIF crosstalk

D Bandarra, S Rocha*

Abstract

Introduction

Hypoxia-inducible factor is a key transcriptional factor involved in the cellular response to low levels of oxygen, hypoxia. Moreover, hypoxia-inducible factor has been recently associated with a role in inflammation and immunity. Importantly, hypoxia-inducible factor is regulated by the major inflammatory responsive transcription factor, nuclear factor- κ B. These two major pathways have been intimately linked. On one hand, they share a number of common target genes; on the other hand, physical interactions between hypoxia-inducible factor subunits and nuclear factor- κ B have been observed. Even though the role of nuclear factor- κ B over hypoxia-inducible factor is fairly well-known, the involvement of hypoxia-inducible factor over the nuclear factor- κ B pathway is not. Given the overlap between these pathways, it would not be surprising to find a functional involvement of hypoxia-inducible factor in processes where nuclear factor- κ B is involved. In this review, we will describe the communalities between hypoxia-inducible factor and nuclear factor- κ B pathways, highlighting the crosstalk that occurs in a variety of conditions.

Conclusion

Taken together all the communalities between hypoxia-inducible factor and nuclear factor- κ B pathways, there is no doubt that a cross-

talk occurs, which can potentially bring new insights for therapeutic intervention in situations of disease such as cancer, stroke or rheumatoid arthritis.

Introduction

Oxygen is essential for multicellular organisms. As such, being able to respond to variations in oxygen availability is a requirement for the survival and homeostasis of the organism. Sensing and responding appropriately to oxygen changes is important for a variety of important physiological processes, which include high altitude living, intense exercise and embryo development. However, lowering of the oxygen concentration or availability (hypoxia) is part and/or contributes to a number of human pathologies, such as cancer, stroke/infarction, diabetes and ageing¹⁻². Understanding the molecular mechanisms controlling the cellular response to hypoxia is, therefore, of great importance. One master regulator of the cellular response to oxygen changes is the family of transcription factors, hypoxia-inducible factor (HIF). However, HIF activity has been associated with additional stimuli that do not involve changes in oxygen, such as infection and inflammation³. These findings led to the discovery that HIF is controlled by a transcription factor, mostly involved in immune responses, nuclear factor- κ B (NF- κ B). In this review, we will highlight the shared features of these transcription factors, from activating stimulus to common targets.

Discussion

The authors have referenced some of their own studies in this review.

The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

HIF pathway

At the molecular level, the cellular response to hypoxia relies on HIF. HIF was first identified in 1995 together with hypoxia response element (HRE, 5'-RCGTG-3') of the erythropoietin gene (EPO). Further studies revealed that HIF is actually a heterodimeric complex comprising an α - and a β -subunit, which exist as a series of isoforms: -1 α , -2 α , and 3 α . HIF-1 α is constitutively expressed, while HIF-2 α and HIF-3 α expression is restricted to a subset of tissues.

Even though HIF-1 β expression and protein are not dependent on oxygen changes, HIF- α subunits are extremely labile at normal oxygen levels. This occurs mostly at the protein level, with HIF- α half-life being very short (~ 5 min), while transcription changes in response to oxygen have not been widely reported thus far.

The activity of the complex HIF-1 α -HIF-1 β is determined by the stabilisation of the α subunit during hypoxia. In the presence of oxygen (normoxia), HIF- α is regulated by a class of dioxygenases called prolyl hydroxylases (PHDs), of which four isoforms have been identified so far (PHD1, PHD2, PHD3 and PHD4). These proteins use iron, 2-oxoglutarate, ascorbate and molecular oxygen as co-factors to catalyse the hydroxylation of HIF- α . The hydroxylation of specific prolyl residues promotes the interaction of HIF with von Hippel-Lindau protein (VHL) containing E3 ligase complex, which mediates proteasomal-mediated

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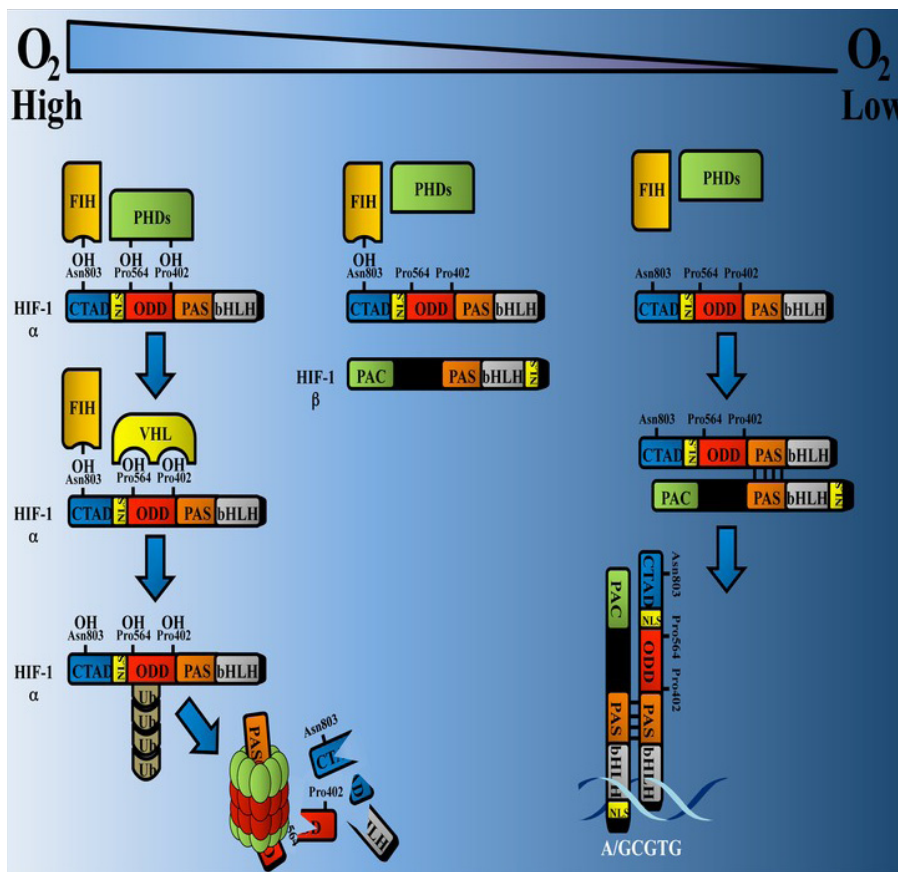


Figure 1: Hypoxia-inducible factor pathway. The HIF system and its regulation are shown in the figure. In the presence of oxygen (e.g. during normoxia), PHDs bind to HIF- α and catalyse the hydroxylation of specific proline residues within the ODD domain (Pro402 and Pro564). Once hydroxylated, HIF- α binds rapidly to the VHL tumour-suppressor protein (an E3 ligase), which results in its polyubiquitination. This targets HIF- α for proteasome-mediated degradation. An extra oxygen-dependent hydroxylation event takes place on HIF- α , which concerns a single asparagine residue within the C-terminal transactivation domain (Asn803). Asparagine hydroxylation is mediated by FIH, and this modification prevents the association between HIF- α and p300/CBP (not shown). In the presence of low oxygen, HIF- α is stabilised and can translocate to the nucleus. HIF- α dimerises with its partner HIF-1 β and transactivates target genes containing hypoxia responsive elements (A/GCGTG). Note: Molecules are not drawn to scale. bHLH, basic helix-loop-helix; CTAD, C-terminal transactivation domain; FIH, factor inhibiting HIF; NLS, nuclear localisation signal; ODD, oxygen-dependent-degradation domain; PAS, Per/ARNT/Sim domain; PHD, prolyl hydroxylases; VHL, von Hippel-Lindau tumour-suppressor protein.

degradation (Figure 1). Thus, when oxygen levels are reduced or any of the PHD's co-factors are not available, there is an increase in HIF- α subunit levels due to inhibition of PHD activity.

An extra oxygen-dependent hydroxylation event mediated by factor

inhibiting HIF (FIH) takes place at the transactivation domain of HIF- α . This hydroxylation prevents the association of the transcriptional co-activators p300/CBP, and consequently HIF activation (Figure 1). FIH-mediated repression occurs at

much lower levels of oxygen when compared to PHD-mediated regulation⁴. This offers the cell an additional level of control over HIF, highlighting the importance of correct control over this system. To date, HIF has more than 100 target genes identified, involved in key cellular processes such as angiogenesis, glucose/energy metabolism and cell growth/apoptosis. Of these, genetic studies have demonstrated that 40% of HIF-dependent genes are also regulated by FIH⁵.

NF- κ B pathway

NF- κ B is the collective name for a family of transcription factors that include RelA (p65), RelB, c-Rel, NF- κ B1 (p105/p50) and NF- κ B2 (p100/p52). NF- κ B is normally held inactive in the cytoplasm by the I κ B family of inhibitory proteins. However, in the presence of a stress stimulus, such as the inflammatory cytokine tumour necrosis factor- α (TNF- α), I κ B is phosphorylated by the I κ B kinase (IKK) complex. This creates a recognition motif for the Skp1-Cul1-F box (SCF)- β TRCP (beta-transducin repeat containing) complex, which promotes lysine 48 ubiquitination and proteasomal degradation. Degradation of I κ B results in NF- κ B dimer release and translocates into the nucleus (Figure 2).

There are three major pathways leading to NF- κ B activation: (1) the canonical, (2) the non-canonical, and (3) atypical pathways (Figure 2). The canonical pathway is activated by external ligands binding to a specific membrane receptor, resulting in the recruitment of a number of adaptor molecules and activation of the TAK1-IKK complex. Upon activation, IKK mediates the phosphorylation of I κ B α at Ser 32 and 36, which signals it for proteasomal degradation. This results in NF- κ B dimer release and translocation into the nucleus.

In the non-canonical pathway, ligand binding results in the activation of NIK, which leads to the activation

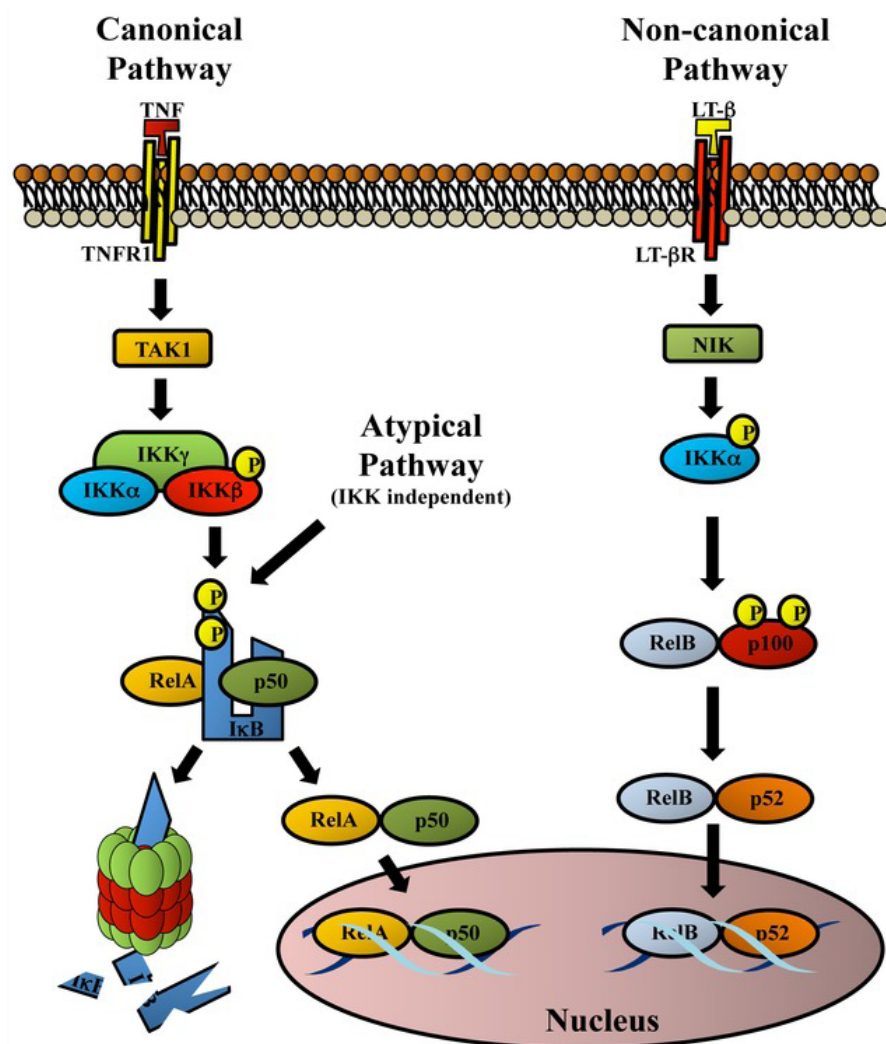


Figure 2: NF-κB pathway. Canonical Pathway: The presence of a ligand, such as TNF-α, results in the activation of TAK1–IKK complex, which mediates the phosphorylation of IκB, which signals it for proteasomal degradation. This results in NF-κB dimer release and translocation into the nucleus. Non-canonical pathway: In the presence of a ligand binding, such as LT-β, NIK is activated, which leads to the activation of IKKα. The activation of IKKα results in the processing of p100 to p52 and binding to RelB. p52/RelB are then able to translocate into the nucleus and activate target genes. Atypical pathway: NF-κB is activated in ligand-independent manner, leading to either IKK-dependent or -independent modes of NF-κB release. In majority of these, IκB is not degraded but dissociation from the NF-κB dimer is possible due to modification of IκB proteins (not shown).

of IKKα resulting in the processing of p100 to p52 and binding to RelB. p52/RelB is then able to translocate into the nucleus and activate target genes.

In the atypical pathway, NF-κB is activated in a ligand-independent

manner, leading to either IKK-dependent or -independent modes of NF-κB release. In the majority of these, IκB is not degraded but dissociated from the NF-κB dimer due to modification of IκB proteins⁶.

All the NF-κB subunits share a Rel homology domain responsible for DNA binding and dimerisation. These transcription factors bind to κB sites in promoters and enhancers of a variety of genes, inducing or repressing accordingly with the cellular context. The specificity of target genes activation is achieved, not only through the combination of the different NF-κB dimers, but also by the formation of complexes with co-activators and co-repressors, and with other transcription factors, such as STATs (Signal transducer and activator of transcription), c-Fos, c-Jun, AP-1, and interestingly HIF⁷. NF-κB regulates several crucial cellular pathways, such as proliferation, apoptosis, angiogenesis and metastasis. It is also known that aberrant activation of NF-κB is associated with many diseases, namely cancer.

HIF and NF-κB physical crosstalk

Several reports have discussed the physical interaction between components of the HIF and NF-κB pathways. HIF-2α was the first HIF subunit to be shown to interact with NF-κB⁸. Here, it was shown that HIF-2α, but not HIF-1α, interacts with the NF-κB regulatory subunit IKKγ (NEMO) *in vitro*. Furthermore, it was also shown that this interaction enhances HIF-2α transcription activity in normoxia.

IκB, p100 and p105 have been shown to be hydroxylated by FIH⁹, and a possible functional crosstalk between PHDs and IKK activation has also been postulated¹⁰. However, the physiological setting for these observations has not yet been investigated.

HIF-1β was also shown to interact physically with NF-κB¹¹. In CD30-stimulated cells, HIF-1β was shown to interact with RelB and p52 subunits. HIF-1β binding to RelB was also shown to be important on RelB bound to NF-κB-responsive promoters¹¹. HIF-2α and HIF-1β are not the only HIF subunits interacting with NF-κB; HIF-1α has recently been

shown to interact with RelA in EGF-induced cells¹². The authors showed that EGF-induced HIF-1 α interacts with RelA, and this interaction is crucial for RelA bound to the PKM promoter, and PKM2 expression. Furthermore, HIF-1 α is a co-activator of PKM2 transcription, and plays an important role in aerobic glycolysis and tumour growth¹².

Thus far, no reports exist to prove interactions between the additional NF- κ B family members and HIF subunits. In addition, whether the reported physical associations are also evident under hypoxia and/or inflammation conditions is also not clear. Furthermore, additional work is necessary to determine if the physical interaction between NF- κ B and HIF subunits is DNA-dependent or if indeed it is the result of protein-protein interactions. Nevertheless, given the reports on the physical crosstalk between HIF and NF- κ B, it is likely that a functional involvement of HIF over the NF- κ B pathway happens, and therefore it will be important to study the physiological relevance of these interactions in a cellular context.

HIF and NF- κ B functional crosstalk, shared targets and activating stimuli

Even though HIF is the central transcription factor in response to hypoxia in the cell, other proteins have been reported to have an important role in the hypoxic response¹³. Several reports have shown that NF- κ B is activated during hypoxia^{6,14}. Our laboratory has investigated the mechanism by which NF- κ B is initially activated in hypoxia; we showed that IKK and TAK1 are induced in hypoxia independent of the molecular oxygen sensors, PHD1 to 3, or HIF-1 α . IKK and TAK1 are activated by a mechanism involving Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), which had been already implicated in response to hypoxia-ischaemia during brain development *in vivo*¹⁵.

Interestingly, we also have revealed a mechanism where I κ B α ubiquitination is prevented by hypoxia, and sumoylation on lysine 21 through Sumo-2/3 takes place, resulting in NF- κ B activation⁶. Sumoylation is not only important in activating NF- κ B, but also to stabilise HIF in hypoxia. It has been reported in mice the role for SENP1, a Sumo protease, a modulator of EPO production by regulating HIF-1 α stability during hypoxia. HIF is sumoylated in hypoxia, promoting its interaction with VHL and consequent degradation through the proteasome¹⁶. Furthermore, HIF-2 α is also a target of sumoylation, which reduces its transcription activity¹⁷. It has recently been shown that VHL is inactivated through sumoylation by a SUMO E3 ligase in hypoxia¹⁸.

Although most of the knowledge regarding HIF has been derived from studies following hypoxic stress, HIF- α stabilisation has also been found in non-hypoxic settings, such as relatively well-oxygenated regions of tumours, and in diseases such as rheumatoid arthritis and diabetes^{19–20}. HIF has been shown to be induced in response to growth factors (e.g. insulin-like growth factor 1 and platelet-derived growth factor), cytokines (e.g. TNF- α and IL-1 (Interleukin-1)), ROS (Reactive Oxygen Species), all of which are activators of the transcription factor NF- κ B^{3,21}. Furthermore, our and other laboratories have shown that NF- κ B is a direct modulator of HIF expression by regulating basal, TNF- α and hypoxia-induced HIF expression^{3,22–23}.

An additional level of functional crosstalk resides in the number of common target genes. Perhaps, the most well-studied gene activated by HIF and NF- κ B is VEGF (Vascular Endothelial Growth Factor), a potent angiogenic growth factor^{24–25}. Apart from VEGF, many important genes are regulated by HIF and NF- κ B (Table 1). These include cytokines and chemokines, such as TNF- α , IL-1 β and IL-8. In addition, cell death

related proteins such as Noxa and BNIP3, and other important cellular proteins such as PKM2, Tert, Cyclin D1, and Cox-2 are also shared HIF and NF- κ B targets. However, it is not known if these genes are targeted by these transcription factors at the same time or independently of each other. Furthermore, whether these genes are co-regulated following a shared activating stimulus is also unclear. Further work is thus needed to answer these important questions.

HIF and NF- κ B functional crosstalk, common regulators

Several proteins have been associated in the modulation of both HIF and NF- κ B pathways. One example is tumour necrosis factor receptor associated factor 6 (TRAF6). TRAF6 is a crucial signalling mediator involved in the regulation of several physiological processes, such as adaptive and innate immunity, development of different tissues, and bone metabolism²⁶. TRAF6 is an E3-ligase for K63-linked polyubiquitination together with the E2 enzyme complex that consists of UBC13 and UEV1A²⁷. The ubiquitination by TRAF6 induces NF- κ B by activating TAK1 kinase, which phosphorylates IKK β leading to IKK activation²⁸. Recently, TRAF6 has been shown to regulate HIF-1 α expression independent of NF- κ B²⁹. TRAF6 was shown to promote K63 ubiquitination of HIF-1 α , which results in increased protein stability and activity²⁹.

Another point of crosstalk between HIF and NF- κ B is through the F-box and WD repeat domain-containing 7 (FBW7). FBW7 is a component of SCF box ubiquitin ligase responsible for targeting several apoptosis-, growth- and proliferation-related proteins, such as cyclin E, c-Myc and Notch³⁰. FBW7 was shown to target HIF-1 α for degradation in hypoxia through a mechanism involving phosphorylation of HIF by glycogen synthase kinase 3 β (GSK3 β), followed by ubiquitination and proteasomal degradation³¹. Furthermore, it has been

Table 1 Shared target genes between HIF and NF- κ B. Here is shown the list of genes and their function, described as targets of both HIF and NF- κ B transcription factors

Gene Symbol	Function
ABCB1	P-glycoprotein-drug resistance
ASPH	Aspartyl-beta-hydroxylase
BCL2L11	Pro-apoptotic Bcl-2 homolog
BNIP3	Hypoxia-inducible death factor
CCND1	Cyclin D1
CDKN1A	Cyclin-dependent kinase inhibitor
EDN1	Vasoconstrictor peptide/mitogen
ENG	Endothelial cell membrane glycoprotein
ENO2	Enolase 2 gamma
EPO	Erythropoietin
FN1	Extracellular attachment
GAD1	Glutamic acid decarboxylase
GADD45B	DNA repair/cell cycle
HMOX1	Hemeoxygenase
IGFBP1	Insulin-like growth factor binding protein-1
IGFBP2	Insulin-like growth factor binding protein-2
IL1B	Interleukin-1 β
IL8	Interleukin-8 α chemokine
IRF1	Interferon regulatory factor-1
KLF10	TGF- β early response gene
MYLK	Myosin light chain kinase
NOS2A	Inducible nitric oxide synthase
NR3C1	Glucocorticoid receptor
PGK1	Phosphoglycerate kinase 1
PIGF	Placenta Growth Factor
PIM1	Ser/Thr kinase
PMAIP1	Pro-apoptotic member of the Bcl-2 protein family
PPP5C	Protein phosphatase 5
PTGS2	Cyclooxygenase
PTPN13	Protein phosphatase
SERPINE1	Plasminogen activator inhibitor
SLC16A1	Monocarboxylate transporter isoform 1
SLC6A6	Taurine Uptake Transporter
TERT	Telomerase catalytic subunit
TF	Transferrin
TFF3	Peptide in response to gut irritation
TFR1	Transferrin Rceptor
TGM2	Tissue transglutaminase
UCP2	Uncoupling protein-2
UGCG1	Glycosphingolipid
VEGF	Vascular endothelial growth factor
VIM	Intermediate filament protein
WT1	Zinc finger transcription factor

shown that FBW7 associates physically with HIF-1 α , and this results in HIF- α degradation, and control of the hypoxia response *in vivo*³². Our own work, in collaboration with Sangfelt's laboratory, has shown that FBW7 interacts directly with p100 via a conserved degron and that it promotes degradation of p100 through the GSK3 β in phosphorylation-dependent manner. This interaction also affects the complex between the active form of p100, p52, and RelB, which consequently changes the apoptotic balance in the cell³⁰. However, what is the contribution of FBW7-mediated regulation of NF- κ B towards the regulation of HIF-1 α is not yet known. Further work will be needed to detangle this intense crosstalk.

HIF protein regulation is mediated by the tumour suppressor VHL. VHL promotes K48 ubiquitination of HIF- α subunits in normoxia²⁵. VHL has also been shown to negatively regulate NF- κ B activity³³ and HIF-independent mechanisms have been put forward³⁴. With further research being conducted, it is very likely that additional common regulators will be identified.

Conclusion

The crosstalk between HIF and NF- κ B pathways is extensive and intensive (Figure 3). From physical to functional interactions in response to many common stimuli, it is possible to speculate that this crosstalk helps coordinate the cellular response adopted by the cell. With the overlap of these common regulators of HIF and NF- κ B would not be surprising to find a functional involvement of HIF in processes where NF- κ B is involved, such as infection and inflammation. Taken together all the communalities between HIF and NF- κ B pathways, there is no doubt that a crosstalk occurs which can potentially bring new insights for therapeutic intervention in situations of disease, including cancer, stroke and inflammatory conditions.

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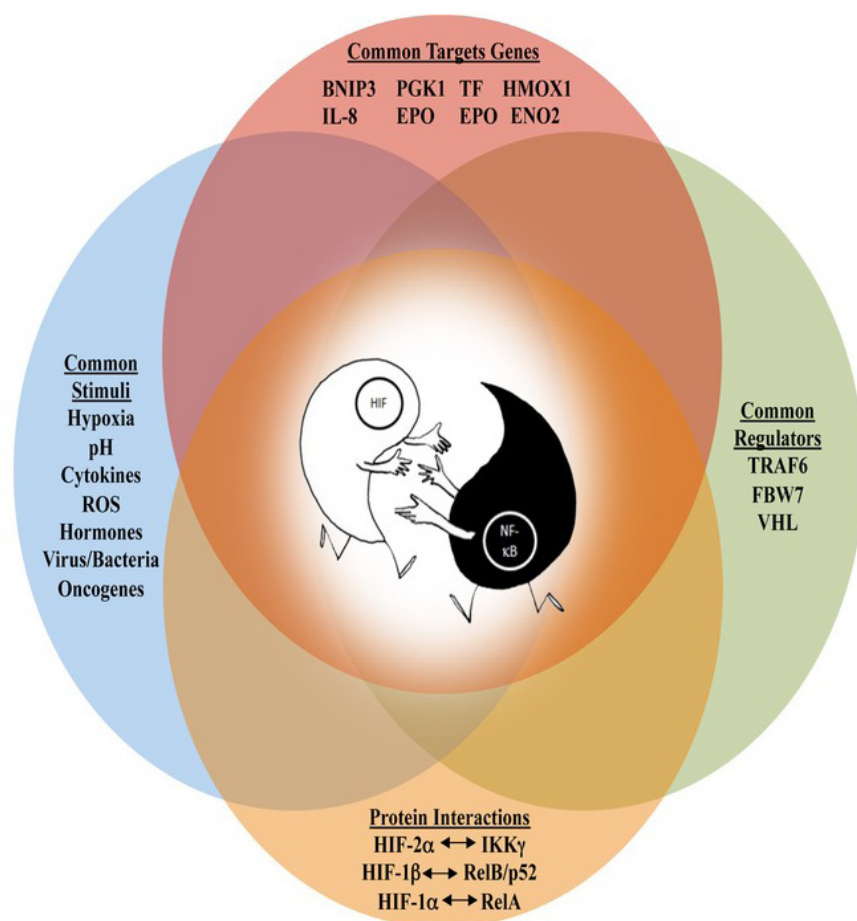


Figure 3: HIF and NF-κB crosstalk. In this figure, communalities between HIF and NF-κB pathways have been shown, which highlight the crosstalk that occurs in a variety of conditions (common stimuli). Some of the shared target genes between HIF and NF-κB (common target genes) as well as some regulators of both pathways, and protein interactions described so far between HIF and NF-κB are also shown.

Abbreviations list

β-TRCP, beta transducin repeat containing protein; CaMKII, calmodulin-dependent protein kinase II; EPO, erythropoietin; FBW7, F-box and WD repeat domain; FIH, factor inhibiting HIF; IL-1, Interleukin-1; GSK3β, glycogen synthase kinase 3β; HIF, hypoxia-inducible factor; HRE, hypoxia response element; IKK, IκB kinase; NF-κB, nuclear factor-κB; PHDs, prolyl hydroxylases; ROS, Reactive oxygen species; SCF, Skp1-Cul1-F box; STAT, Signal transducer and activator of transcription; TNF-α, tumour necrosis factor-α; TRAF6, tumour necrosis

factor receptor associated factor 6; VEGF, Vascular endothelial growth factor; VHL, von Hippel-Lindau.

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